

SI Appendix for

Regional control of histone H3 lysine 27 methylation in *Neurospora*

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SI Materials and Methods

cDNA preparation mRNA was purified from 100 µg of DNase-treated total RNA using the Oligotex mRNA Mini Kit (Qiagen Cat. # 70022). First and second strand cDNA synthesis was then conducted on 100 ng of mRNA with random primers using the SuperScript® Double-Stranded cDNA Synthesis Kit (Invitrogen Cat. # 11917-010). The cDNA was fragmented by sonication with a Branson Sonifier 450 on output 1.2, duty cycle 80, for 80 pulses. The fragmented cDNA was purified on Qiagen MinElute columns (Cat. #28206).

Preparation of ChIP-enriched DNA and double-stranded cDNA for sequencing ChIP-enriched DNA and double-stranded cDNA were prepared for sequencing by first blunting the ends of the fragments using the Quick Blunting Kit (New England Biolabs #E1201L) followed by purification of the DNA on a Qiagen MinElute column using the PCR purification protocol. Adenosines (A's) were then added to the blunted ends using Klenow fragment (3' to 5' exo-) (New England Biolabs #M0212S) followed by purification of the DNA on a Qiagen MinElute column using the reaction cleanup protocol. Paired-end adapters containing 6 bp barcodes, to allow for multiplexing, were ligated to the DNA fragments with Quick DNA Ligase (New England Biolabs #M2200S). The DNA was size-fractionated on a 2% agarose gel. "Invisible" fragments between 250-400 bp were excised and purified with the MinElute Qiagen Gel Purification system (Qiagen Cat. # 28604). Purified DNA was then amplified by PCR using the PfuTurbo Cx Hotstart DNA polymerase (Agilent Technologies Cat. #600410) using a limited number of cycles. Amplified DNA was size-fractionated on a 2% agarose gel in 1xTAE buffer. The "smear" of amplified DNA between 300-450 bp was excised from the gel and purified as before with the Qiagen MinElute Gel Purification kit. Purified DNA was quantitated using a Qubit fluorometer (Life Technologies), multiplexed with other samples containing different barcodes, and sequenced on either Illumina's Genome Analyzer II (40-nt read length) or HiSeq 2000 (50-nt read length) next-generation sequencers (Genomics Core Facility, University of Oregon).

Sequence analysis Sequence alignments were performed using Bowtie with default settings (1) and output in SAM format (2). The SAMTools tool kit was utilized to convert from the

SAM format to the BAM format and to remove PCR artifacts (rmdup tool) that appeared as large spikes in the data (2). RNA-Seq reads were mapped to the *N. crassa* OR74A reference genome using the default settings on TopHat (3). The mapped RNA-Seq reads were then used to estimate transcript abundance (FPKM - fragments per kilobase of exon per million fragments mapped) using the Cufflinks program (3). The Cuffdiff program was used to compare the relative abundance of transcripts between different RNA-Seq samples (3). For display purposes, ChIP-Seq and RNA-Seq reads were processed using the "count" function of igvtools (<http://www.broadinstitute.org/igv/igvtools>) to generate a tiled data file (.tdf) representing read densities over 300 bp windows across the genome. These .tdf files were displayed using the Integrative Genomics Viewer (IGV: <http://www.broadinstitute.org/igv>) (4).

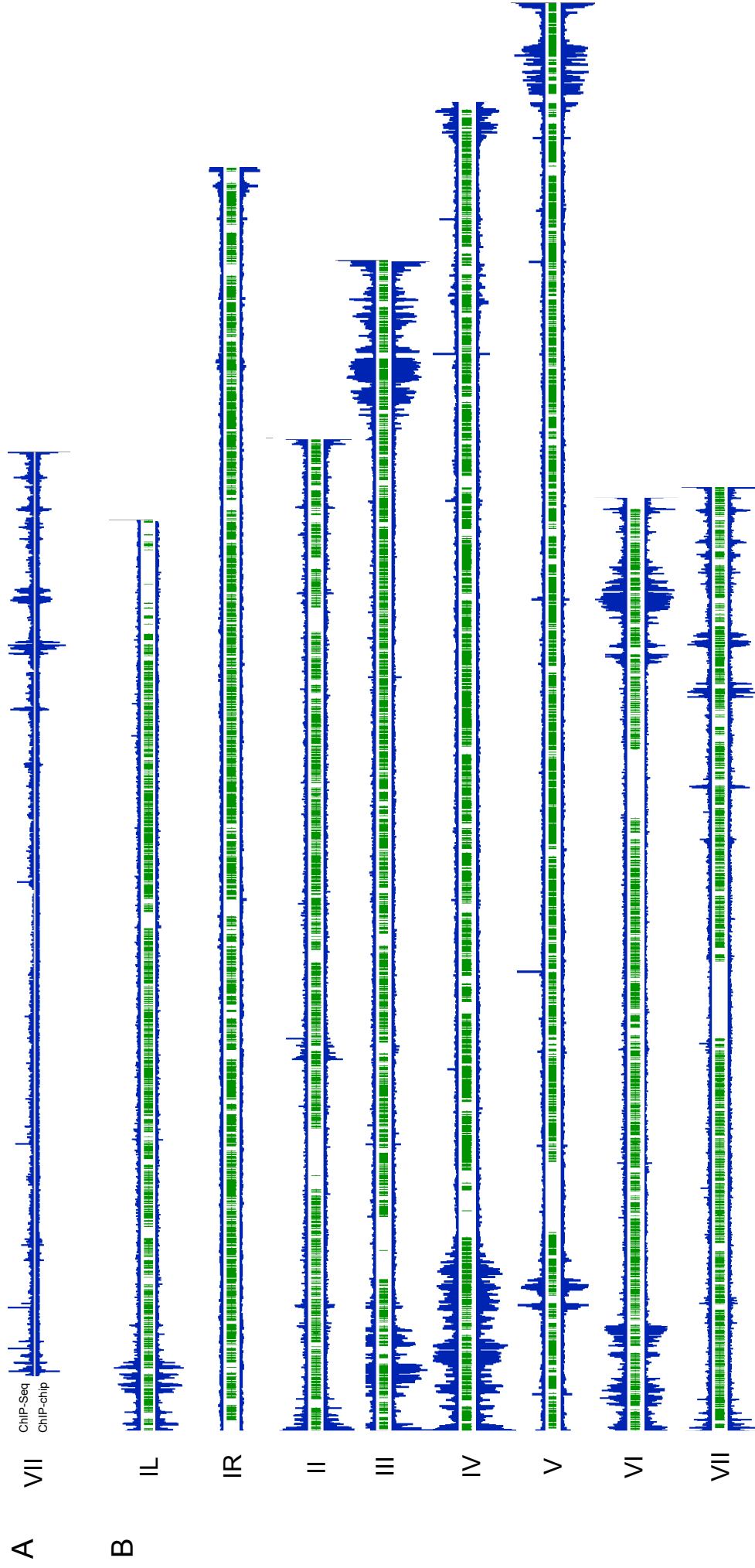
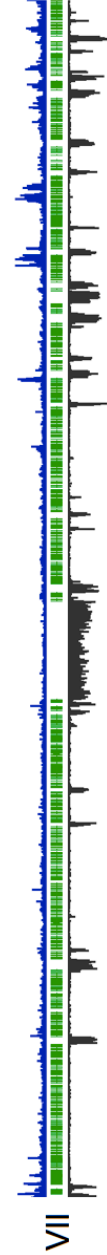
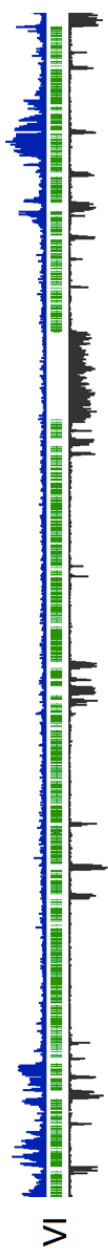
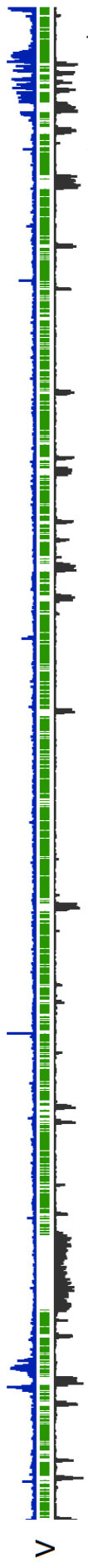
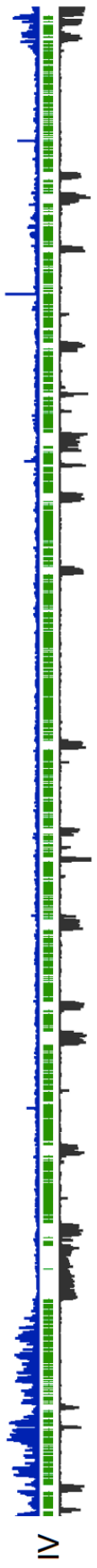
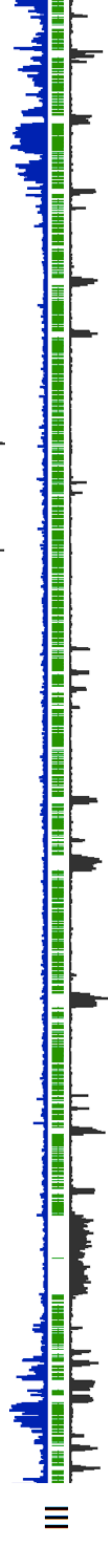
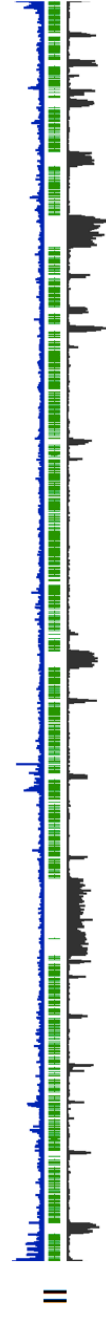
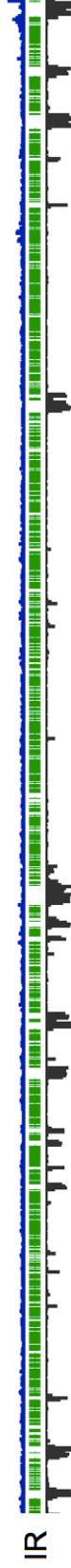
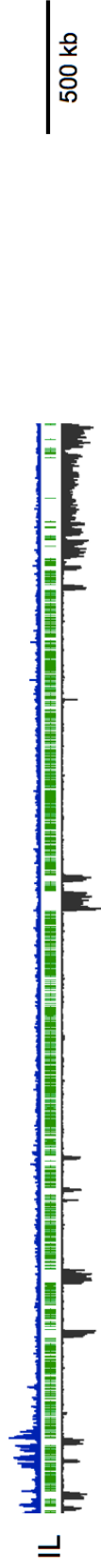


Fig. S1. H3K27me3 domains are reproducibly detected. (A) The H3K27me3 ChIP-Seq read density profile (upper track) closely matches that obtained by ChIP-chip for LG VII (lower track). (B) H3K27me3 ChIP-Seq read density is shown for the seven LGs of *N. crassa* for cultures grown in Bird's medium (blue above the genes) and in Vogel's medium (inverted, blue below the genes). Virtually identical profiles were obtained.

A



B

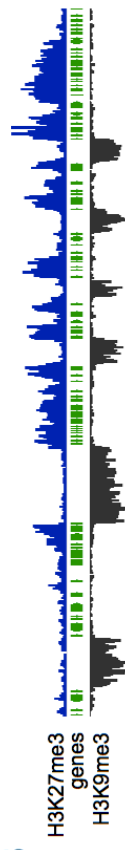


Fig. S2. Genome-wide distribution of H3K27me3 and H3K9me3 in *N. crassa*. (A) Predicted genes (vertical green lines), distribution of H3K27me3 (blue traces above genes) and H3K9me3 (black traces below genes) are represented to scale on the seven LGs of *N. crassa*. The largest chromosome, LG I, is divided at the right end of its centromere into IL and IR. (B) A portion of the right arm of LG V near the telomere is expanded to detail mutually exclusive H3K27me3 and H3K9me3 domains.

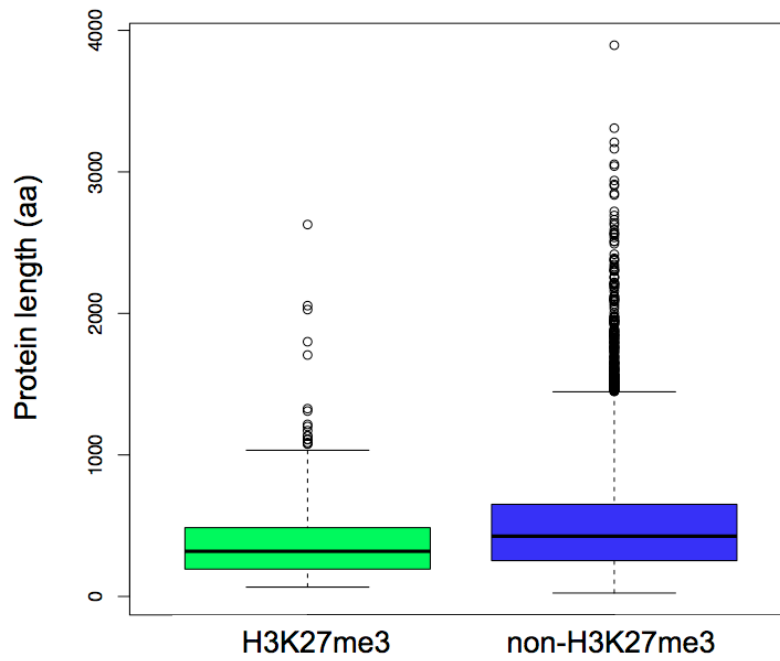


Fig. S3. Predicted lengths of proteins encoded by H3K27me3-marked and unmarked genes of *N. crassa*. Box-and-whisker plot of protein lengths show a significantly shorter average length for H3K27me3 marked genes ($P < 2 \times 10^{-16}$, t-test).

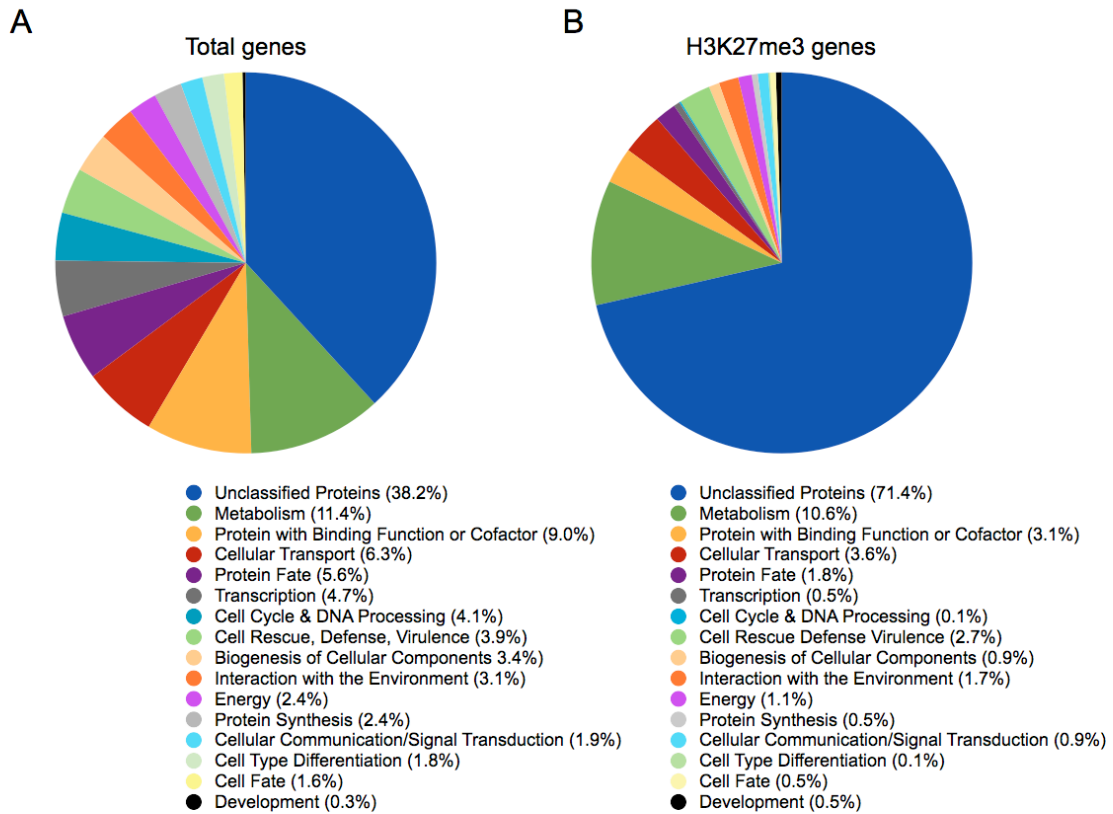


Fig. S4. Functional Category (FunCat) classification of *N. crassa* H3K27me3 genes. (A) Pie chart displaying the FunCat classification of all *N. crassa* predicted genes. (B) Pie chart displaying the FunCat classification of *N. crassa* genes found within H3K27me3 domains.

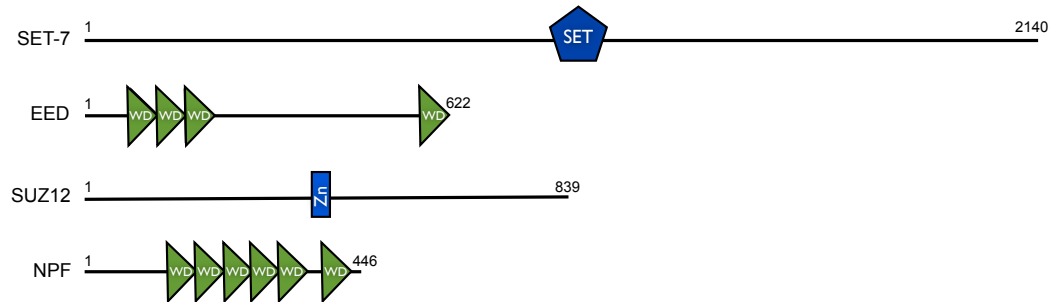


Fig. S5. Domain structures of the four core subunits of the *N. crassa* PRC2 complex. SET-7, the closest *Neurospora* homologue of *Drosophila* E(Z), contains the presumptive histone H3K27 methyltransferase domain (SET; blue pentagon); EED and NPF each possess multiple WD40 repeat domains (green triangles); *N. crassa* SU(Z)12 has a presumptive zinc-binding domain (blue rectangle).

A

Neurospora_crassa	MPVPDPFSLADRTDESSKADGSSSDDEDDGVVVRANAPTEGITFNIGSIHYREDLNP SLGRGRGLLWKR-PTIIPRQPSPVQVVIIRKSSSPAKTTTTTTTT	100
Mus_musculus		
Drosophila_melanogaster		
Arabidopsis_thaliana		
Caenorhabditis_elegans		
Neurospora_crassa	TSTSNHHGSGTKINNRTGSGSDSDSGDSSSSSSKVNSS-GTSRSKKTINDRETTTPPTQPKPTTVTKTMFKPTTSTQDPDEKPPAQVHDEEPRKSYH	200
Mus_musculus	-----MGQTGKKSEKPCVCRW-----KRWKSEYMLRLQLKRRFADEVKMTESNRQKILERTETLN-----QENKQRRIQP	67
Drosophila_melanogaster	-----MNSTKYVPFWK-----RRKSEYIKIQOQKRYKRADEIKAEWIRNWDHNNHVNQDLY-----CESKRVQAKP	62
Arabidopsis_thaliana	-----MASEASPSSSATRSEPPKDSAEERGSPASKESEVIELSKLLAADRCISIKKRIDENKKNLFAITQSFM-RSSMERGGSCCKDGSLLLVKRODSP	95
Caenorhabditis_elegans	-----MSNSEPTSTPTSGTKTKRKGKCTETSMGSKSKSNLPRFVKIQPIFESSEKIKETVCEQGI-----ECKRMLK	67
Neurospora_crassa	PLLPKPVFGPA-RKTACGTAPSRVPEGHHISASQGSFKPMVGSIFRPPSQTAPKAAPFQLONSHRSYSQSOPOLHNTSSS-ISSSKPPLKTAIQTAPGQG	299
Mus_musculus	-----VHI-----MTSVSSLRGTRCEVTSDDLPFAQVIFPKLTNAVASVPMYSSWSPLOQN-FMVEDETVLHNT-----	130
Drosophila_melanogaster	-----PHVDC-VKRAEVTSYNGIPSGQKVPICVINAVPTIPMTYTWAPTOQN-FMVEDETVLHNT-----	123
Arabidopsis_thaliana	-----GMKSGIDESN-----NNRYVEDGPASSCMVGQSSVPVKISLRPIKMPDIK-RLSPYTTWVFLDRNQRMTEQSSVGGRRRIY-----	170
Caenorhabditis_elegans	-----GHF-NAIKDD-----YDIRKDELDOTDIKDWLKDASSSVNEYYRRRLQENLCEGRTIAKFSFKNCEKYEENDYKSDSTV-----	140
Neurospora_crassa	QRPTTPPPPVSSSVFKPHQPPRQPSQVPPGICQSOPAPDMPQPLASVT-LPPQPOQQQQQQQQPQPSRPOPQOPQPPQQRSTSKFISAPVASSSSK	379
Mus_musculus	-----IPYMGDEVLD-----QDQTFIE-----ELIKNYDGKVGHDR-ECGFINDEIFVELVNALG-----	199
Drosophila_melanogaster	-----IPYMGDEVLD-----KDGKFI-----ELIKNYDGKVGHDR-DPSFMDDAIFVELVHALMRSYSKLEEEAAPGTATAI	190
Arabidopsis_thaliana	-----YDQTGGEALICDSEEEAIDDEEEKRDFLEPEYIIRMTL-EQLGLSDSVLAELASFISRSTSEIKARHG-----VLMK	243
Caenorhabditis_elegans	-----TWIKPDRTE-----EGLDMK-----KFRAPCSRIEVGDISPPMIYVWPIEQSVATPDQLRLTHMP-----	195
Neurospora_crassa	VNSQEPATQPPFAPNLTQVD-IESKLSFIATVCEDHARFVEYLDEAEQMAPEPKHLSDFAFADMPALSAPATTSDTASISDDGVETMA-FKIKLHHG	498
Mus_musculus	-----QYNDDD-----DDD-----DGDDPD-----EREKQKDL-----DNRDDK-ETCPR-----	216
Drosophila_melanogaster	-----KTPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Arabidopsis_thaliana	-----KTPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Caenorhabditis_elegans	-----KTPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Neurospora_crassa	DNGKPRAPTKAFKCPVVKIKTDEVEVP-KYRFHHTIEKKNIIVPNTMLTFVPHLRDVPDPSVD-ERDYSIWLNELEKLDQTQCFTEENROQKNHNRVDF	597
Mus_musculus	-----KFPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Drosophila_melanogaster	-----KFPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Arabidopsis_thaliana	-----KFPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Caenorhabditis_elegans	-----KFPADKIFEAISMPFDKGTAE-ELKEKYELTEQQLGALPECTPNIDGNPAKSV-----REQLHS-FHTLCRCFCFYDCLHPHFH-ATPNT	358
Neurospora_crassa	TATLSMYIEPWLKGLDVCGRPTLIRYMSLQ-EENKAHITQQQKDVLLNTYKDDAIISSKAVEAARIFTLAFNNVFGNNTDPERFITLRDVLLEKRET	697
Mus_musculus	-----YK-----RKNTEALDNKP-CGQCQYHLEGGKAEFAALTAERIKTPPKRP-GRRRRGLRPNNSRPTPTIIVLESKDTSDREAG	385
Drosophila_melanogaster	-----YK-----RKNTEALDNKP-CGQCQYHLEGGKAEFAALTAERIKTPPKRP-GRRRRGLRPNNSRPTPTIIVLESKDTSDREAG	385
Arabidopsis_thaliana	-----YK-----RKNTEALDNKP-CGQCQYHLEGGKAEFAALTAERIKTPPKRP-GRRRRGLRPNNSRPTPTIIVLESKDTSDREAG	385
Caenorhabditis_elegans	-----YK-----RKNTEALDNKP-CGQCQYHLEGGKAEFAALTAERIKTPPKRP-GRRRRGLRPNNSRPTPTIIVLESKDTSDREAG	385
Neurospora_crassa	VVD-EKRAKETPPANPQRDQSDSNGLLPKVEASLSYAVLGCNVCFSHDCHEGDI DAHNYHRTFLSDSVGGVI-RALKRQWADQVASMGGDEEAVAAASK	796
Mus_musculus	-----TET-----GGENNDKEEEKDSTSS-----	406
Drosophila_melanogaster	-----TET-----GGENNDKEEEKDSTSS-----	406
Arabidopsis_thaliana	-----TET-----GGENNDKEEEKDSTSS-----	406
Caenorhabditis_elegans	-----TET-----GGENNDKEEEKDSTSS-----	406
Neurospora_crassa	KALHLPCHNACYRHYDVGPAAPVTPWANSEISVLEDMFVSUGH-SQTLKACQVVASI LGRKCEWYVRKIKELDLS-----LP	873
Mus_musculus	-----SSEANSRCQTP IKM-KPNI EPPENVEWSGAEASMFVRLIGTYD-NFCAIARL-----IGTKTCRQVYEFVRKES-----I	477
Drosophila_melanogaster	-----VAEINSIMAGMMNI-----TSTQCVMTGADQALYRVLHVVYLG-NYCAIAHN-----MLTKTCRQVYEFQAQEDA-----E	492
Arabidopsis_thaliana	-----GSDSELRKEEFMGETVSRGLATNKLWRPIELSLFDKGVEIFGM-NCSL IARN-----LLSGFKSGWVFQYMTCSSENKASF GGGDGLNPDGSSKFDI NGNMVN	696
Caenorhabditis_elegans	-----VDEDEDEESKIRS-----CRDAYHLLMSMAENV SARL LKMGQPS-ARLSIKDRVNNFRNQLSQEAKAKRLRHDSL-----	400
Neurospora_crassa	QVSPRPRTKPGKGGPPTKVKPLPWYDRKKKCLMGDWQ-DQATTHEHSIREITEPCHHDDGCTKE-----NEACPANASPRPLLCDR-FCQCTVDEGALKT	968
Mus_musculus	-----APVPTEDVDTPPR-KKKRKHRLWAACHRKILQKK-----DGSSNHVYNYQPCDHPRQPCDSSQPCV-----IAQNFCEK-FCQCSS-ECONRFP	558
Drosophila_melanogaster	-----FEDLRQDFTPPR-KKKRKHRLWAACHRKILQKK-----DGSSNHVYNYQPCDHPRQPCDSSQPCV-----IAQNFCEK-FCQCSS-ECONRFP	558
Arabidopsis_thaliana	-----FEDLRQDFTPPR-KKKRKHRLWAACHRKILQKK-----DGSSNHVYNYQPCDHPRQPCDSSQPCV-----IAQNFCEK-FCQCSS-ECONRFP	558
Caenorhabditis_elegans	-----FEDLRQDFTPPR-KKKRKHRLWAACHRKILQKK-----DGSSNHVYNYQPCDHPRQPCDSSQPCV-----IAQNFCEK-FCQCSS-ECONRFP	558
Neurospora_crassa	RIQALRDGLDAEKLIEDDMRDSQRNEXRMTA-----VTPITACRHAGCNAT-----AENGAAR-ENG-VGSY-MCKGDI-NCQRFP	567
Mus_musculus	-----CAACHSTGKTCI-QRQEGKPGCICIMLNRECDPVVCKGEGAKERADPNDAHDET LHSTGQNVSLQRGASKTVLLGKSQLEGC-GYGLTAE-DI SODEFV	1066
Drosophila_melanogaster	-----GC-RCKAAGNT-----KQCPYLAVERCDPDLGLTEGAADHWDK-----NVSKNCSIQRGSKRHLL-----APSDVAGWGIIFIKD-PVQRNEFI	638
Arabidopsis_thaliana	-----GHEAKSQERS-----RQCPFAADRECDPDERNEWVIGCGCS LGVP SQRGDNYERNNMLLLKQQRVLL-GISDYSWGAFLKN-SVSKHEYL	652
Caenorhabditis_elegans	-----GHEAKSQERS-----RQCPFAADRECDPDERNEWVIGCGCS LGVP SQRGDNYERNNMLLLKQQRVLL-GISDYSWGAFLKN-SVSKHEYL	652
Neurospora_crassa	TEYTGELITHDEGVRREARRGEGFGSGTSSYLFTLLEHEGIIWYDAAMYGNLSR-YINHASENDKACNITPKI IYNNNEYRIKFTALRDIKAGEELFFNY	1166
Mus_musculus	-----SEVCGEISODE-----ADRRKQVYDKY-MESFLNLNN-DPVVDATRKGNKIRF-ANHSV-----NPNCYAKVM-MVNGDHRIGIFAKRAIQTEELFFDY	726
Drosophila_melanogaster	-----SEVCGEISODE-----ADRRKQVYDKY-MESFLNLNN-DPVVDATRKGNKIRF-ANHSV-----NPNCYAKVM-MVNGDHRIGIFAKRAIQTEELFFDY	726
Arabidopsis_thaliana	-----SEVCGEISODE-----ADRRKQVYDKY-MESFLNLNN-DPVVDATRKGNKIRF-ANHSV-----NPNCYAKVM-MVNGDHRIGIFAKRAIQTEELFFDY	726
Caenorhabditis_elegans	-----SEVCGEISODE-----ADRRKQVYDKY-MESFLNLNN-DPVVDATRKGNKIRF-ANHSV-----NPNCYAKVM-MVNGDHRIGIFAKRAIQTEELFFDY	726
Neurospora_crassa	GDNFPNLTKKLLLEDQDGDGENDTA-TKSGKGRGSSLAQGTARKATTKASTTAKGAKTQGRAGARKTAVMEIPP SDDYEDQWTWRDPLPYDE-YDEDD	1265
Mus_musculus	-----RISQADALRYVG-----RISQADALRYVG-----IEREIMEIP-----	746
Drosophila_melanogaster	-----RYCPTQLKIFYG-----RYCPTQLKIFYG-----IEREIMEIP-----	760
Arabidopsis_thaliana	-----RYEPDRAPAWAKPEAPGSKK-DE-----NVTPSVGRPKKLA-----	902
Caenorhabditis_elegans	-----SKSGEHQIAFRMQTKERSKPSR-----PKSQKLSKPMPTSE-----	773
Neurospora_crassa	DSYLPVGRKKRRKGRGAGRAGKRRKTPSPEEGEEEGEDHGSAGEDDAEAEAEAGDEDDGGDANG-P SNQQRNRRTAVSEISDSQAERDEDDMESESD	1365
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	SDAPLSPTRTVATARRRRQLRTTAAQTTTTTTTTTAA-I NNTSRATSSASASAYTTAASTNS	1465
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	TPAP S-OPCGGGCGDGDDEAEGNEEQEEEEEGQRKKRSNRGARGPAGRKPRKMGQQAASSTASVSASETTA-AGSHAGSYWDRLLSSIAFGSGSGS	1564
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	SSGPAQLANPHSGSLPANPGSISPSKSKRKS SKRRKPAEAEIYSM-AEYSSSGFGSGSDAELFSAPESKGDHASP SKKKKQKTSTSTSTSTTTTAAANT	1664
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	RTRTTTRSRSARATAA-KTTTSPATMKIKPLGVTVTRSPGGRTGAARHTSMHNAAAQAQLRAEQSLRDEAAAAAAGAGASNAQ-PQRLAQCVQVQCE	1763
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	MQATQGGGQQMQGSTSSGLYHTAANFQPTFSNDDEDDGEEDGASVSGSGEEEEE-EEDEEEEEEEEEEEEEEGEDGASLASGEEEEEQGNRKLGL	1863
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	SGEEDDEEDDEDDGSDGSDGDDGLDV-DVEHGDADAYTYTYAENDLLLSLSNEEEGILGDYSDGAASNP SGPESGSSNEGDESDDDDDDDDTGG-DR	1962
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	EESKEEDQSPVKRLRPHPHQASPTTKSMASKARPPVVGKVLQRSSSQSSQSQSQPHSQSKITRQ-SSTATGTRSTERKITRSTNTSGTTSTRKPSNS	2062
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	
Neurospora_crassa	NTNTNPKLKP KPGSPSKETRSTAAASVQNNPTRGSS-SSLKRRKASGRAGSTGGGDMEGTSHRKRQRLRYRNEE	2140
Mus_musculus	-----	
Drosophila_melanogaster	-----	
Arabidopsis_thaliana	-----	
Caenorhabditis_elegans	-----	

B

Neurospora crassa -----MPTNKAP-----TSNEWDLKLRAS-----INFQDDWKDPVSGESIAEHFFDVK 44
Mus_musculus MSEREVSTPAGTDMPAAKKQLSSDENSNDPLSGDENDDAVSIESGTINTERPDTPTNTPNAPGRKSWGK-GKWKSK-KCKYSFKCVNSLKE-DHNQPLFCVQ 100
Drosophila_melanogaster MS-----SDKVNKNENPEEESGCGDE-----SASYTNTSTSRKSKSPSSSTRSKRRGRRST-----KSKPKSRAAYKYDVHVKENHGANIFCGA 80
Arabidopsis_thaliana -----KITLGNE-----SIVGSLTSEN-----KKSYSKYVTRNIQEE-GKKPLYAVW 401
Caenorhabditis_elegans -----MEHTKKFKS-----LNHGFDRSTEDYG-----KRPFLVITAKLLE-DQKKAIYGLA 45

Neurospora crassa TYPYNPVGAPPVFAISKKHVICTRI-NQNTDSTNPVEVLKLRD-----DDDDAANCSCCKSKDMETGQ-----PWLCTAGADAKVKYVDVKGKGL-V 132
Mus_musculus FNWHSKEGDPVLFATVGSNRVTLYEC-----HSQGE-IRLLQSYVD-----ADADENFYTCAWTYDSNTSH-----PLLAVAGSRGIRIINPITMQC-I 183
Drosophila_melanogaster FNTLLGKDEPQVFATAGSNRVTVEYEC-----PROGG-MQLLHCYAD-----PPDEVFYTCAWSYDLKTS5-----PLLAAAGYRQVIRVIDVEQNEA-V 163
Arabidopsis_thaliana FNFLLDAR-FFDVFYTAGGNRITLNC-----LDGGA-ISALQSYAD-----EDKEESFYTVSHACGVNGNP-----YVAAAGVYKIRVIDVNSETI-H 121
Caenorhabditis_elegans FNQYAGIDEEQAVATVGGSFLLHMS-----VPIDINNELQWCSNFPDTKSSKVERESLFTVTCYDTYEAENDRNPFKVVGTGLGHVYVDVSRKL-S 141

Neurospora crassa KTLVGHGG-GINDLVTSPLT-PSLIASCS-DDTIVRLWSLPLIHSAPQEMFIFLG--GDAHTWLLSIAFHDTGR-YLLSAGHDQ-TINLWITPPCSEPVTHP 228
Mus_musculus KHYVGHGN-AINELKFHPRD-PNLLLSVS-KDHALLRLWNIQ-----TDTLVAIFG-GVEGHRDEVLSADYDLLEGE-KIMSCGMDH-SLKLV-----263
Drosophila_melanogaster GNYIGHQD-AINELKFHPRD-LQLLLSVS-KDHALLRLWNIQ-----SHVEIAIFG-GVEGHRDEVLSIDFNMRGD-RIVSFCGMDH-SLKLV-----243
Arabidopsis_thaliana KSLVGHGD-SVNEIRTPQ-LK-PQLVITAS-KDESRLWNVNE-----TGCEILIFA-GAGGHRYEVLSDVDFHSPDIYRFASCGMDT-TIKIW-----202
Caenorhabditis_elegans NLRLSVW-EIDIRTCAN-SNLIVCAS-SQSIIRIHHIR-----NEAELIVIG-GLCHAGTILSDVWSTDGDFIL-SCGFH-QLMEV-----221

Neurospora crassa LVIIHPHFSTKEIHNSLVDCVSFFGDLISRACWEEFVLWVSISGFS55555-FPSPSSSPSS55555FQGNHLLPSTAPTTFDPKSLTRSAFWQAPDLNIFETRP 330
Mus_musculus -----RINSKRMMNAIKESYDY-NPNKTNRP-----FISQIKHFDPS-300
Drosophila_melanogaster -----CLNTPFHHKIELSNTF-SQEKSTLP-----FPTVTXHFDPST-280
Arabidopsis_thaliana -----SMK-EFWTY-VEKSTFW-----TDDPSK-----FPTKFVQFVFT-235
Caenorhabditis_elegans -----DL5VKQVKEHLERACKALHQQINVLTQSQDIPYVSKGTMKRSVAVS-----RNIPDKEEDQLLE-LHRELIIPRPSCLLIYTP 298

Neurospora crassa AYFTRLLQFKTVDKGGQFYM-RFKILHAQCGKHPVLAENARNKFMFLSLRSGWQFELGELRDAEEQEAEKNLKEGGRGCRKRVRVE-APGWMPARKMP 431
Mus_musculus -----TRDIHRNYDGVNRWLG-DLILSKS-----EDDIKIKP-----345
Drosophila_melanogaster -----TRDIHRNYDGVNRWLG-DLILSKS-----GEN-AIVCWPKGQL-----HQSFEOVKP-----325
Arabidopsis_thaliana -----ASIIHNYDGNRWFG-DFILSKS-----VDN-EIILWEP-----QLKENSP-----274
Caenorhabditis_elegans SSVSTDMHSDYVDGIRFLIGTNYALSKG-----CGNEKAIHFWRFGPP-----KGEVENRIH-----350

Neurospora crassa RRTAGATGEGGGGGTTSSTAAAAATTAANYNKTKGQOQOQLCTGTS-TSPDPETLLSASAVAAAAAGGPRGSSSRERF-GSRGCTTTT 532
Mus_musculus -----SESNV-TILGRFDYSG-----DIWYMRFSM-----DFWQKMLALQNOVKLYLVVWDEVEDPHAKKCTTETH 406
Drosophila_melanogaster -----SDSSC-TIIAEFYDCE-----DIWYRFGF-----NPWQKVIALNQGGKYVWELDPSDEGAHMTLHN 386
Arabidopsis_thaliana -----GEGASD-VLLRYPVPMG-----DIWFIKFS-----DLHLSSVAIQNGEKKYVWDLKSCPP-VLITKLKSH 333
Caenorhabditis_elegans -----GNVLRPKSCTKFRTMNVPSCG-----SAWFIKFAV-----DPRRRVLVCGGAGGSMVFDFLRNNEE-TNPTHTCS 414

Neurospora crassa ATATATATATAASRAPGICLGGYTKQL-DEWHQCDITDSHKEIKPHKTYTVDGKGQHFVGRQVAVSPEGNWCVVVGNRNRAFIQRWGK 622
Mus_musculus HKCGAAIRQTSFSR-DSSILIAVCDASIRWRDRLR-----441
Drosophila_melanogaster SRSVATVQGLAISR-DASVLYVYCDATVWRNRR-OTTSI-----425
Arabidopsis_thaliana NQKSVIRQTAMSDVGS-TLACCEDGTIRWWDVITK-----369
Caenorhabditis_elegans VCSRTV-RQASFSTCGR-FVLVLVTEGFCVCRFDVRSASVDKDLAKF-----459

C

Neurospora crassa MGKNPSTQSRVPAHESDVRFDHSHKPDQDRDDTCQHPAKRRRLNDNHG-----FPLYEDPNGNTPRCIRIEVLK-IHKD 75
Mus_musculus ---MAPOKHGGGGGGGSGPAGCGGGCGGSAAGAAASCGKSGGGGCGGGGYSASSSSAAAAAAGAAV-LPVKNKMEHIVADHLEFQAFKPTQIYR- 100
Drosophila_melanogaster ---MAFAKREKSDNPDGSAANGILGLTHGAPDASAGSTVPTAEG-----QVKLNCHQEQELFQAFKPTQIYR- 70
Arabidopsis_thaliana -----MPGILPLVSRETSSECRSTEQMCHESRLRISEE-----EEIAAESAAAYCKPVELXN- 53

Neurospora crassa SPVKNKNGPNGIAPNVRDANMRACKLSYGHNGDEVLVLDQGLCELKVYRNPAASTHMA-RLYAIKAFDIPEDKIYMERGSDPAFGADYSVLEIE 179
Mus_musculus FLRTNRLIAPILHRTLTYSMSHRNRTSIRKRTFKVDML-SKEKMKGEQE--SHLSAHLQLT---FTGFHKNDK---PQSNSENE---QNSVTLEVLV 191
Drosophila_melanogaster LIRNRHETNPILNRTLSYMKERMSNNKRIISQVNSMLESITQKSEAVSQNYLHVYDLSLEK---LPARL-DNESGEDLLQEQLLCEA---GESSVETTLV 168
Arabidopsis_thaliana IIQRAIRANPLFGLQCL-HYKIEAKHKRRIQMTVFLSGAIDVGTQKLFPLYLILARLVSPKPV---AEYSAYVRFKACILTGLCGVDG-VSQANFLPLD 153

Neurospora crassa SAGDPNWPSPKLPKAVEDPLVRNLPQRQA-LYAQLPDIHTRNKRTRVAKRVQSRPTDFVMDIDVRWQTSISSQLTGN---QPKRIMP---S 272
Mus_musculus KVCHKRRKDVSCPITQVPT-KKKQVP-----LNPLDNLQTKGNPFLAVSSNEFEPNSNHMVKSYSLLFRVTRPGRRFNGMINGETNIDVSE-ELPAR 285
Drosophila_melanogaster KITRSKRKDSITDFQELLS-KCSQIV-----YNP---KDRVGEHTISIP-L-QTMRPMGEQHTLYKLLFRIKVLSPTCN---DENAETTP---246
Arabidopsis_thaliana NRLALEKSGSLAIFISFAGAONSQ-----FGIDSGIKHNGIGHCLWS---KIPQLSLYASW-QKSPNMDLQQRVD---VSLVEMP---232

Neurospora crassa TVIDPWAP-KPLSELPLVNGTNGVKGINGHHDPNTSNEIRKTNWNEVNSTSLADTEELAEGLDTPNRSRRHRTVEVNYN-VRQWNTAVGEPKRRRRADDHP 375
Mus_musculus RKRNRDEGKETFAVQMTVFDKNNRQLLDGEYEVAM---QEMECPISKKRATWETILDGKR-LPPFET-FSQGPTLQ---FTIRWTGETNDKSTAPVAKPLATR 382
Drosophila_melanogaster NKRSRPN-EKMGSELILYEKSS-GFITEGEYAML-QPLNSTSISFSPKKCTWETMPDSYIPLSLTYDVYQSPMLK---FHUTLS---NEQLPEMISAPLELR 343
Arabidopsis_thaliana ---CFIKLKSMEEEKCVSIQVPSNPLTSSPQQVQVTISAEVGGSTKSPY-SFSYNDISSSLQIIRLRTGNVVF--YRYNNKLQKTETVEDFSCLFCLV 331

Neurospora crassa LDHEHTVYVLPETPGNEE---TLQATCNKLSCLICAAEHDRISQLRA-HFSCHPYEYFNEFQKKGMVLAHVHRACNTSTMTMPLEASKIFSLGLPVKRLDLSK 476
Mus_musculus NSELHQENKYSYVPAQDT---IAVKETLTLEOTRKEKN-SNESROKLRIFYOFLYNNNTROQTAEADDLHCWCTLNE---RKLVSLLKHLKCHSFR 476
Drosophila_melanogaster YVQHLDPA-VAEMNNNNY---NNNNCSGLKNGSGGNSCTVCTTPEHIOIVNMYNSNTRQQTETYTELLNCPWCCLOC---LRLYALBKHLKCHARF 437
Arabidopsis_thaliana KCASFGLRHLPSSTHDLN-NFEFWYTEEFAQVNVSLKETETMISKVNEDDVPKQQTFFSRRRQKSQVRSSROGHCLGCG-----EVLDKTD 419

Neurospora crassa FVNGDSDVWKSRLP-DPNDCEP-PHLPASRPTKGPQQQQQQQQQLQQQQQQHQHQKESIPTTSTRSAAAAATTKPQKKPVY---IPHNINRPIDYPL 574
Mus_musculus IFNYYHFKPGARLD-VSINECYDGSYAGNPQDIHQPGGFASR-NGPVRKPTIT-HILVCRPKRTKASMESEFLESEDEGEQRTYS-SGHNRLYFSDTCLP 577
Drosophila_melanogaster NFYIQPAGSGARID-VITNDAYDGSYAGSPYDLAGPSSGFSARTCGNTRSVT-SLMVCRPRKQKTCLEDFELEDEDEISNQSMTI-TGHNRLYHETETCLRVH 539
Arabidopsis_thaliana D-AHSVRSEKSRIPCKHYERICGASEQ-RVPPGTSPADVQSCGDVYVYIAGSTMQLQFAKTRKISIERSD-LRNRSLQKQKFF---HSTHAQPMAL 514

Neurospora crassa KVELAPGSEVYRPLTEGWLITKH-ADALGSEVDPEQKEKYMMOWDAYILQKHLCS-EQYLPREFRNVREKATWLLKRSRAEELGKHMAYILAR-RYVDAT 676
Mus_musculus PQEMEVED---EKDPEWLREKT-ITQIEEFSVDNEGEKELNKLWNLHVMHKGFI-ANQMNHACMLFVNYGQKIIKK-NLCRNFMLHLSMHDF-NLISIMS 675
Drosophila_melanogaster FKELIDSEG---ESDPEWLRLQKT-IQMIDEFSDVNEGEKELNKLWNLHVMHKGFGV-DCQLPIACEMLDAGKTEIVRK-NLYRNFILHMCSLFDY-GLIAAET 637
Arabidopsis_thaliana QVLSRDSSEG---EVDVADVED-RRMLDDVYDTKDEQMHMNSFW-RKQVRLADGHIPWACEASRLHGPIMVTRPHILICWVRVFMVKLWNH-GLDART 613

Neurospora crassa YMAVTKEN-----EARKMAAAEISANGP-EGAD 704
Mus_musculus IDKAVTKLR-----EMOQKLEKESATPSN-EAIEA 705
Drosophila_melanogaster YKTVKQGLGLSKYAAQGLMQQRQEELKYWLDVGMHKKQEDPTKLSPQKPAAPPADQAST-SASTSGSGSGSSMQPPKRMPLAHLKRGSAASPQVQSGKT 741
Arabidopsis_thaliana MNCNTFLEQLI-----626

Neurospora crassa AQAQSKESAQQQQQQQQQSSGRKKSAAAGCARCGEVVPQPEMVICCNKPMRAKPGRGAPTSEQA-EVEVLQVRSSGPLEVAQAQSRQSQSRDQSGKMLQQT 808
Mus_musculus EGNCKNKGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGKLETSGLGK 808
Drosophila_melanogaster ENGTNGSNNSSSNKNAKKSADQPLSTL-ANTERRSEYQGKRNVSGRLAATPASKRKLSSKNDTVLNKRQRYSDGSPGTGIGNGHGGGSGGANRNK-SNNH 844
Arabidopsis_thaliana -----844

Neurospora crassa FTRTARSERYEPGCVSVYQDATILTSLH-----839
Mus_musculus -----900
Drosophila_melanogaster SLPATSNNA55555SNKRAIARRRSTERTKASGSGGGAGGVRTRLSVPAYERR-----900
Arabidopsis_thaliana -----900

D

Neurospora crassa MARDEIVDDVNMEDDAEAQRLLINEFYKWKNSFLYDMLLSTALEWRTLTQWFVDKPKDKSH-TVHRL--LGT-HTAEG-----KPNYLQIEVE 95
Mus_musculus -----MTLKSSEEGCGSMRTALSDLYLHLLORRRP-----ETSLNQSNVTEDMYTNGSPAGPSAHAKGQAEARRV-LIQFEKITEPMGMITLKNEKQSCSTVARIL 100
Drosophila_melanogaster -----MYDRSDNAAESFDAAVEERVINEEYKWKNSPTFLYDLVMTHALWSPSLTAQWLPDVKQDGKDY-SVHRL--LGT-HTSO-----EPNHLIAVSG 86
Arabidopsis_thaliana -----MGDEEMRGEIERLLINEFYKWKNSPTFLYDLVMTHALWSPSLTAQWLPDVKQDGKDY-SVHRL--LGT-HTSO-----EPNHLIAVSG 86

Neurospora crassa HP-KMVELNPRDYDEERGEICGGYSGASSGEPICRFKIKTKIDHP---GEYNKARYQPONPDI-IATLAVDGRVLIFORTKHSITR-SGTPSQLEILGHEGFE 196
Mus_musculus IGMCIHROGSLHVDEILEINCTNVNTH-----SVDOQLKAMKETN-KMISLKVIANQSRALPALQMFMRQFDYDPQKNLIPCKEAGLKFVTGDIIOIKNDKS 200
Drosophila_melanogaster LPSDAQFGSHYDNEKGEFGGFGSGVG-----KIEIEIKNH---GEYNKARYQPON-ACVIAKTPSDVLVFDYTKHPSKRPESGCGDPLRIRGHQEGY 186
Arabidopsis_thaliana LPLDDTSEARQYDORRSGFGFGCATG-----KIEIEIKNH---GEYNKARYQPONFII-IATVTYNAEYVFDYSKHPSKRPLDAGNPLDKHGLSME 183

Neurospora crassa LNNNPHGEC---LVTE-SEKTVLLWD-LKTYEGTSKOLKYSRKYTHSHLVND-WOHPLVKSWIGTVSDBLTIOIBVRKPEIDKAA-IVARNCHSDAI-NAIA- 295
Mus_musculus NNWQGRVSS-SKESAGLIPSPLEQERVASVHASAPSEACSCPGTKHKKKMLKHLAKHSSIFDQDVSYEEVVRLPAP-KRRTLVLLIG---QSGVGRSHKNGLS 304
Drosophila_melanogaster LSWNNPLNGY---LSSA-SDDHTICLWD-INATPKEHVRIDAKNIFGTHTAVVED-VAWHLLHESLFGSADDOKMLWDTRNNNTSKPS-HTVDAHTAE-NCLIS- 284
Arabidopsis_thaliana LLSKFKFGH---LSSG-SDDAQICLWD-INA-TPKNKSLDQAQIFKAHFGVVED-VAWHLLHREYLFSGSCDDQYLLIWLDRSPSASKPV---QSVVAHSMVE-NCIA- 280

Neurospora crassa FNRRVETIATASADKICGIDWMMNMKSKVNTLGHQDAVTSLEWHR-----TESALGCS-GSYDRLILFWB-ISRV-GDEQTDAAE-DGPFELLIMH---SGHN 390
Mus_musculus HNRE-KAEPAYPTTRPPKKSEEDGKYHFISTEEMTKNISANFLE-FGSYGNNMFMTGKFETVQIHOKBKIAILDIPDETLLKTVRTALSLFPIAPDQDQOTE 410
Drosophila_melanogaster FNYSFEILATGSADKTVALWDLRLNKLKLSFESHKDEIFQVQWY---HNETILAS-SGTDORRLHWD-LSKI-GEQSTDAE-DGPFELLFIH---GGHTA 379
Arabidopsis_thaliana FNFNFNVVATCTSDTKTVKLDLRKLSLALHTFDSHKEEYFGVGNR---HNETILAS-CCLGRRLMWD-LSRI-DEEQTVDAE-DGPFELLFIH---GGHTS 375

Neurospora crassa HLADESWNRRNDPWLCSAAEDNLQIKWVA-NSIVSKEPDMSTPELDDPKFKQSSH 446
Mus_musculus ALQLOKDS-EAIRSQYAHFDLVNNSVDETLKKLOEAFDOACSSPQWVRVSWY 466
Drosophila_melanogaster ISDFSWNPNEPWIICSVSEDNIMQVQMAENVYNDDEEPI-PASELETNTA-----430
Arabidopsis_thaliana KISDFSWNPCEDWISVSAEDNLQIKWMAENIYHDEDDAP---CEEPSKAS-----424

Fig. S6. Multiple alignments of the PRC2 subunits. The protein sequence from each of the *N. crassa* PRC2 subunits was aligned to corresponding homologues from *Mus musculus*, *Drosophila melanogaster*, *Arabidopsis thaliana*, and *Caenorhabditis elegans* using ClustalWS. (A) Alignment of *N. crassa* SET-7 with homologs. ClustalWS alignments from *N. crassa* (XP_965043), *M. musculus* (AAH16391), *D. melanogaster* (NP_524021), *A. thaliana* (AEC07449) and *C. elegans* (O17514). The SET domain (V1040 to L1173, E = 3.38e-29) is indicated by the black line. (B) Alignment of *N. crassa* EED with homologs. ClustalWS alignments from *N. crassa* (XP_962071), *M. musculus* (NP_068676), *D. melanogaster* (AAA86427), *A. thaliana* (AEE76418) and *C. elegans* (Q9GYS1). Four WD40 domains are indicated by the black lines (S77 to D125, E = 7.96e+00; Q128 to S168, E = 1.74e-08; A175 to T216, E = 1.01e-04; E575 to Q618, E = 2.38e+01). (C) Alignment of *N. crassa* SUZ12 with homologs. ClustalWS alignments from *N. crassa* (XP_963451), *M. musculus* (AAH64461), *D. melanogaster* (Q9NJG9) and *A. thaliana* (AED96057). The zinc finger domain is indicated by the black line (L403 to H425, E = 2.12e+01). (D) Alignment of *N. crassa* NPF with homologs. ClustalWS alignments from *N. crassa* (XP_960994), *M. musculus* (AAC52970), *D. melanogaster* (AAF55146) and *A. thaliana* (AED97021). Five WD40 domains are indicated by the black lines (R130 to D169, E = 3.21e-01; T180 to D220, E = 1.02e-05; Q230 to D270, E = 9.94e-01; T276 to D317, E = 5.86e-06; M321 to D361, E = 2.14e-08; P378 to K418, E = 4.48e-02).

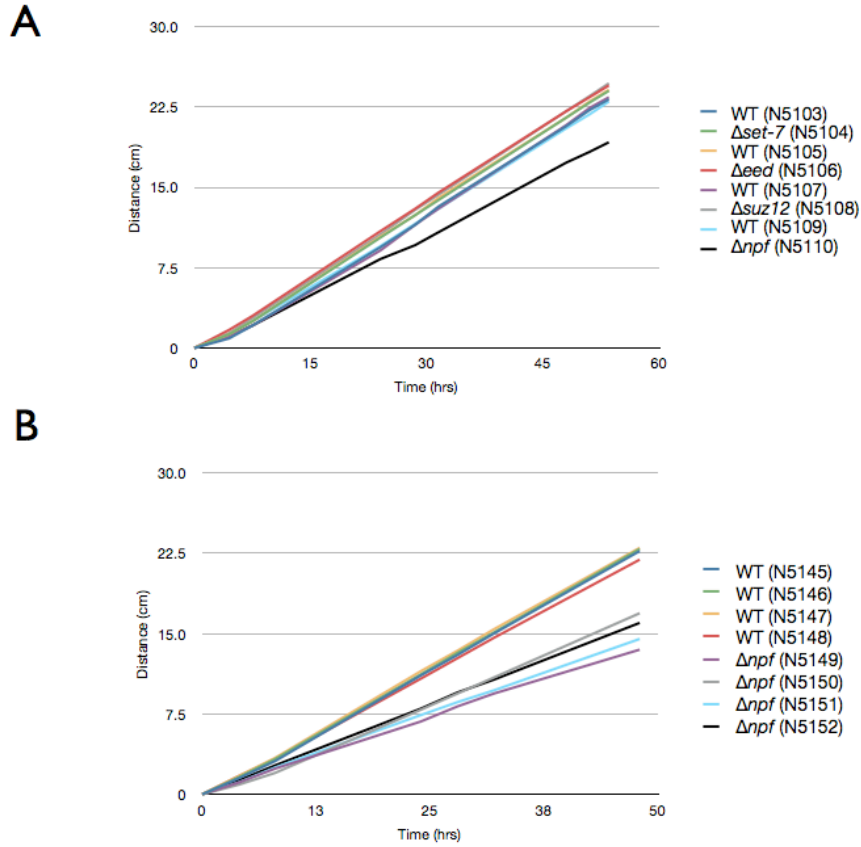


Fig. S7. Linear growth rates of PRC2 subunit deletion mutants. (A) The linear growth rate for wild-type and PRC2 deletion mutant strains (Table S5) was measured by growth in race tubes on Vogel's solid medium (5). (B) The linear growth rate was measured for four wild-type and four Δnpf strains (Table S5) on Vogel's solid medium (5).

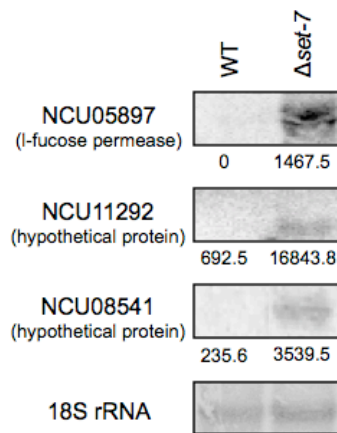


Fig. S8. Increased expression of H3K27me3 genes in the $\Delta set-7$ mutant. Northern blots show the increased expression of three additional genes (NCU05897, NCU11292 and NCU08541) in the $\Delta set-7$ strain. 18S rRNA stained with methylene blue is shown as a loading control.

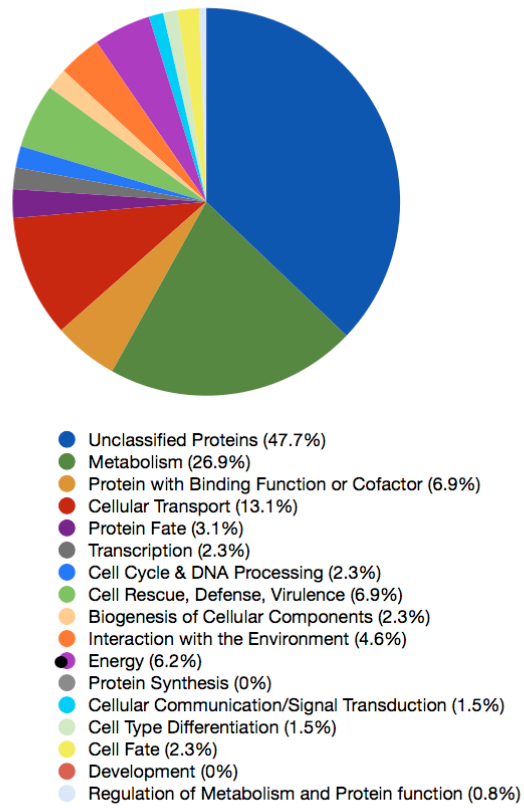


Fig. S9. Functional Category (FunCat) classification of genes showing increased expression of 130 upregulated genes in the $\Delta set-7$ strain.

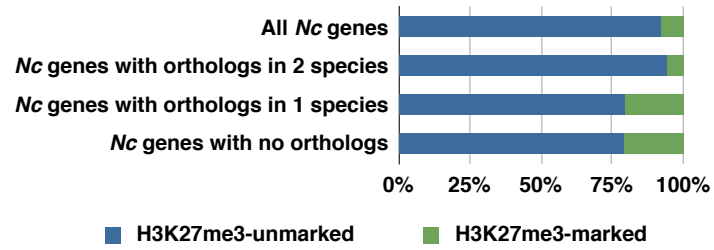


Fig. S10. Proportion of *N. crassa* H3K27me3-marked and –unmarked genes relative to their conservation in two other *Neurospora* species, *N. tetrasperma* (*N.t.*) and *N. discreta* (*N.d.*).

Table S1. H3K27me3 domain analysis summary in *N. crassa*

	H3K27me3 domains	Avg. Domain Size (kb)	Largest Domain (kb)	Total Genome Coverage (MB)	Genome Coverage (%)
WT (Bird's)	223	12.5	107.0*	2.8	6.8
WT (Vogel's)	232	12.1	107.5*	2.8	6.8
Δnpf (Vogel's)	187	8.4	79.5*	1.6	3.9

*The same domain on LG III. RSEG analysis carried out using a bin-size = 500 bp

Table S2. H3K27me3 domain analysis in *Neurospora* species

Species	H3K27me3 domains	Avg. Domain Size (kb)	Largest Domain (kb)	Total Genome Coverage (MB)	Genome Coverage (%)	H3K27me3-marked Genes
<i>N. crassa</i>	223	12.5	107.0	2.8	6.8	774
<i>N. tetrasperma</i>	167	10.8	101.5	1.8	4.6	536
<i>N. discreta</i>	186	14.1	94.5	2.6	7.1	822

RSEG analysis carried out using a bin-size = 500 bp

Table S3. H3K27me3 status of *N. crassa* orthologs in *N. discreta* and *N. tetrasperma*

<i>N. crassa</i>	<i>N. discreta</i>	<i>N. tetrasperma</i>	Gene Count
U	-	-	242
U	-	U	413
U	-	M	5
U	U	-	123
U	U	U	7927
U	U	M	11
U	M	-	8
U	M	U	186
U	M	M	21
M	-	-	66
M	-	U	32
M	-	M	78
M	U	-	8
M	U	U	85
M	U	M	44
M	M	-	25
M	M	U	136
M	M	M	258

Table S4. Purification of EED. An *N. crassa* strain bearing 3X-FLAG tagged EED at the amino-terminus and expressed under the *qa-2* promoter was used to purify the PRC2 complex. Associated proteins were identified by mass-spectrometry and the percent coverage of PRC2 members is indicated.

	MW (kDa)	Coverage (%)
SET-7 NCU07496	175.78	9
EED NCU05300	67.23	66.4
SUZ12 NCU05460	93.33	17.6
NPF NCU06679	50.41	74

Table S5. List of Strains

Experiment	Strain #	FGSC #	Species	Genotype
ChIP-Seq, qChIP, RNA- Seq, Northern blot	N3752	2489	<i>N. crassa</i>	<i>mat A</i>
ChIP- Seq, qChIP, RNA- Seq, Northern blot	N4718	11182	<i>N. crassa</i>	<i>mat a; Δset-7::hph</i>
qChIP	N4719	14852	<i>N. crassa</i>	<i>mat A; Δeed::hph</i>
qChIP	N4720	12769	<i>N. crassa</i>	<i>mat a; Δsu(z)12::hph</i>
ChIP- Seq, qChIP	N4721	13915	<i>N. crassa</i>	<i>mat a; Δnpf::hph</i>
ChIP- Seq	N5012	2508	<i>N. tetrasperma</i>	<i>mat A</i>
ChIP- Seq	N5014	8579	<i>N. discreta</i>	<i>mat A</i>
Race tubes	N5103	---	<i>N. crassa</i>	<i>mat A</i>
Race tubes	N5104	---	<i>N. crassa</i>	<i>mat A; Δset-7::hph</i>
Race tubes	N5105	---	<i>N. crassa</i>	<i>mat A</i>
Race tubes	N5106	---	<i>N. crassa</i>	<i>mat a; Δeed::hph</i>
Race tubes	N5107	---	<i>N. crassa</i>	<i>mat a</i>
Race tubes	N5108	---	<i>N. crassa</i>	<i>mat a; Δsu(z)12::hph</i>
Race tubes	N5109	---	<i>N. crassa</i>	<i>mat a</i>
Race tubes	N5110	---	<i>N. crassa</i>	<i>mat A; Δnpf::hph</i>
Race tubes	N5145	---	<i>N. crassa</i>	<i>mat A</i>
Race tubes	N5146	---	<i>N. crassa</i>	<i>mat a</i>
Race tubes	N5147	---	<i>N. crassa</i>	<i>mat A</i>
Race tubes	N5148	---	<i>N. crassa</i>	<i>mat A</i>
Race tubes	N5149	---	<i>N. crassa</i>	<i>mat A; Δnpf::hph</i>
Race tubes	N5150	---	<i>N. crassa</i>	<i>mat A; Δnpf::hph</i>
Race tubes	N5151	---	<i>N. crassa</i>	<i>mat a; Δnpf::hph</i>
Race tubes	N5152	---	<i>N. crassa</i>	<i>mat a; Δnpf::hph</i>

Table S6. List of primers

Experiment	Name	Sequence
qChIP	Gene 1 NCU06955 FP	GTCTTCGGGCATGGGTATAA
qChIP	Gene 1 NCU06955 RP	GATCAATCCTCTCGACTGGG
qChIP	Gene 2 NCU09590 FP	AGCATCCTCCACTGAGCACT
qChIP	Gene 2 NCU09590 RP	TCGAGTTTGGTAAGTGCTGTT
qChIP	Tel 1L NCU10129 FP	AGCGTTCAAATGCCGTGACCTGT
qChIP	Tel 1L NCU10129 RP	AGTCCAATGGTGCTAACGGCGA
qChIP	Tel 1R NCU10130 FP	GACGGACCTCTTCCGCTCGC
qChIP	Tel 1R NCU10130 RP	CCCTGCACGAGACGGTTCGA
qChIP	<i>hH4</i> NCU01634 FP	CATCAAGGGGTCATTAC
qChIP	<i>hH4</i> NCU01634 RP	TTTGGAATCACCCCTCCAG
Northern probe	NCU08907 FP	CTCACCACCCTCCTCGCCCTCGCC
Northern probe	NCU08907 RP	CCTCAAGCAGCACACAAATCCAAC
Northern probe	NCU05897 FP	CTATGGCCTCGGCGCCCTTCTCGCG
Northern probe	NCU05897 RP	GCCATTACAGGCCCTTCTCGCCGAC
Northern probe	NCU08541 FP	GCAATCAAAATGTCCGTCAACCGC
Northern probe	NCU08541 RP	GACTTGCAATGAGCCCTCAAGCC
Northern probe	NCU09663 FP	GTCGAGGCCGCGCCTCCGTCTCC
Northern probe	NCU09663 RP	CTAGAAGAGACCAAGACCCATACC
Northern probe	NCU11292 FP	CGCTAGCAATATGGCAGGCAAACCG
Northern probe	NCU11292 RP	CCATCAACCTAAGCTTTCGATTCCC
HT-seq	PE-top adapter	5'-ACACTCTTCCCTACACGACGCTCTTCCGATC-barcode-T-3'
HT-seq	PE-bottom adapter	5'-P-barcode-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG-3'
HT-seq	PE barcode #1	TAACCC (top adapter) / GGGTTA (bottom adapter)
HT-seq	PE barcode #2	TAAGGG (top adapter) / CCCTTA (bottom adapter)
HT-seq	PE barcode #3	TCAGTC (top adapter) / GACTGA (bottom adapter)
HT-seq	PE barcode #4	TCGCGC (top adapter) / GCGCGA (bottom adapter)
HT-seq	PE barcode #5	TCTTTCC (top adapter) / GGAAGA (bottom adapter)
HT-seq	PE barcode #6	TGCCGG (top adapter) / CCGGCA (bottom adapter)
HT-seq	PE barcode #7	TGTGTG (top adapter) / CACACA (bottom adapter)
HT-seq	PE barcode #8	TCCTTG (top adapter) / CAAGGA (bottom adapter)
HT-seq	PE barcode #9	TCACAG (top adapter) / CTGTGA (bottom adapter)
HT-seq	PE barcode #10	TGGTTC (top adapter) / GAACCA (bottom adapter)

List of SI Datasets

Dataset S1. H3K27me3 domains determined by RSEG (Bird's Medium)

Dataset S2. List of H3K27me3 genes (Bird's Medium)

Dataset S3. H3K27me3 domains determined by RSEG (Vogel's Medium)

Dataset S4. H3K27me3 domains in *N. tetrasperma* determined by RSEG

Dataset S5. H3K27me3 domains in *N. discreta* determined by RSEG

Dataset S6. H3K27me3 domains in the Δnpf strain determined by RSEG

Dataset S7. Differential domain analysis (RSEG-DIFF) comparing the Δnpf strain to wild-type

Dataset S8. List of genes upregulated in the $\Delta set-7$ strain (H3K27me3 genes in red and border genes in orange)

Dataset S9. List of genes down-regulated in wildtype vs. the $\Delta set-7$ strain

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